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Department of Entomology and Institute of Ecology,
University of Georgia, Athens, Georgia, U.S.A.

A simplified gelatine embedding technique for sectioning litter and soil samples

T. R. Seastedt, Aruna Kothari and D. A. Crossley, Jr.

With 3 figures

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1. Introduction

The importance of determining structural and functional relationships of soil biota within a systematic framework has been recognized by a number of researchers (e. g. Anderson & Healey 1970; Richards 1974; Satchell 1974). Inspection of in situ microflora and fauna is a useful preliminary step in evaluating the roles of various groups within the soil milieu. Anderson & Healey (1970) described a technique for embedding wet soil samples with agar by vacuum impregnation. Their methods produced undisturbed, thin sections of litter and soil suitable for a variety of studies. However, their technique employs specialized or expensive equipment for sample preparation (e.g. special corer, embedding tank and rotary slicer) which prevents the method from being more widely used in research and teaching.

In this report we have simplified Anderson & Healey's methods for both embedding and sectioning soils. All equipment used is present or readily available to most laboratories. The sectioning device presented is suitable for sectioning gravelly soils. Soil sections prepared by these procedures are useful in both visual confirmation of quantitative analyses of soil flora and fauna as well as in formulating new hypotheses for biota-substrate interactions.

2. Methods

2.1. Soil core preparation

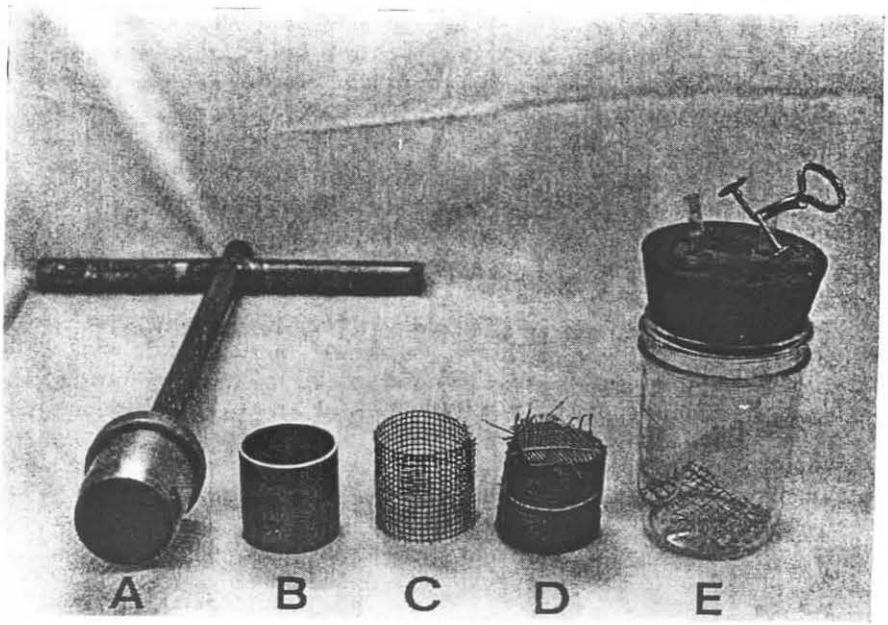
The coring tool used here has been described by Merchant & Crossley (1970) and is illustrated in Figures 1a, 1b. Wire mesh cylinders (Figure 1c) were substituted for metal inserts normally used (e. g. Macfadyen 1962; Merchant & Crossley 1970) in the coring tool. The wire mesh later serves to hold the sample during embedding. Rectangular pieces (15 × 5 cm) of 9 squares per cm mesh screen were rolled into cylinders, fastened by elastic bands, and fitted tightly to the inside of the coring tool.

Litter and soil samples were obtained following the procedure of Anderson & Healey (1970). Dry ice was placed on the site to be sampled to immobilize surface and litter arthropods. After several minutes, the coring tool was placed over the site and gentle pressure applied to hold the litter in place. A sharp knife was used to cut leaf litter along the outline of the cutting edge of the corer prior to pushing the coring tool into the soil.

Samples inside the wire mesh cylinders were capped with circular pieces of wire mesh and wrapped in aluminium foil. These were then placed on dry ice for several hours before being transferred to a freezer.

2.2. Embedding procedure

A 20 percent gelatine solution was used as an embedding medium. Granular gelatine was dissolved in warm water over a flame and placed in an incubator at 45 °C. Soil samples were warmed to room temperature and placed horizontally in a wide-mouth jar (Figure 1e). The hot gelatine solution was poured down the side of the jar until the sample was immersed. A large volume of gelatine solution (250 ml) relative to the volume of the ample (90 cm^3) prevented dilution of gelatine by soil water. The jar was fitted with a rubber stopper and placed in the incubator. A vacuum line was inserted



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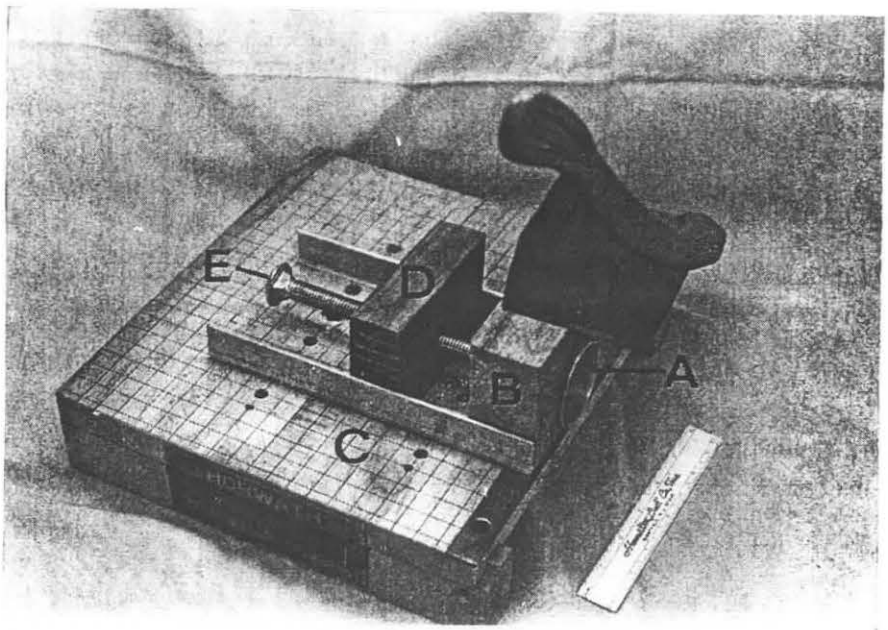

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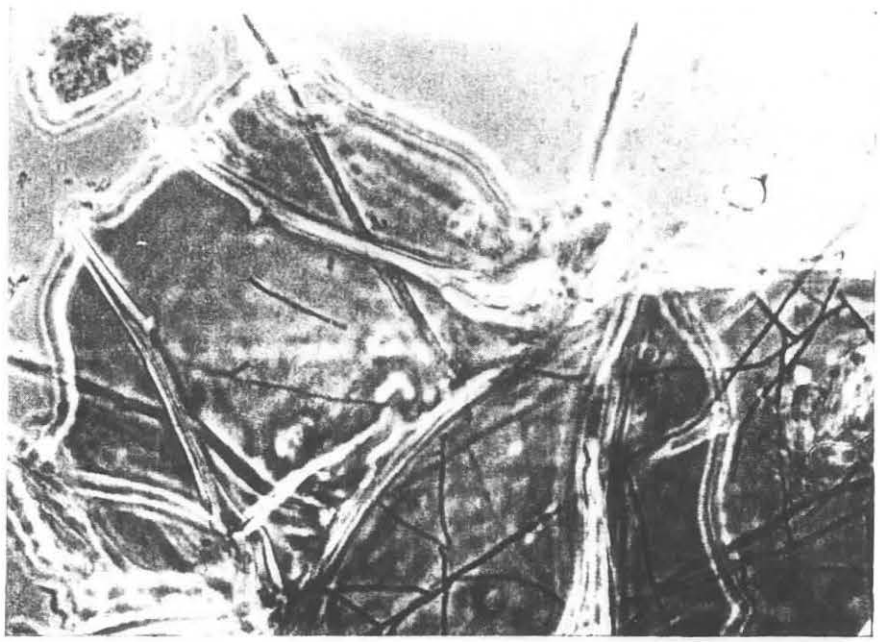

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through a hole in the incubator and fitted to the rubber stopper. The vacuum pressure was increased over a 15 minute period to about 20 cm mercury and maintained overnight at 45 °C. The vacuum was released and reapplied a minimum of 4 times during the first hour. This process helped remove air bubbles from the sample and was done slowly to prevent disturbance of soil structure. Embedded cores were refrigerated until the gelatine was firm and subsequently hardened in 10 percent formalin for a minimum of 5 days.

2.3. Sectioning

A sectioning device was constructed from a paper cutter, a piece of 1.6 mm hardened steel, an aluminium insert such as those normally used in a coring tool, and several small pieces of wood and metal (Figure 2). The apparatus is similar in many respects to that of Haarlov and Weis-Fogh (1953); however, in our apparatus the steel blade combines characteristics of a microtome with those of a meat-cleaver and can therefore section much larger samples. The blade cannot be permanently damaged by sectioning cobbly soils such as those obtained from the Southeastern United States.

The sample is held firmly in place by the metal inset (2a), which is held by a wooden block (2b) mounted to an aluminium frame (2c). A second block of wood (2d) holds a push-rod (2e) which controls the thickness of the section to be sliced. A round disc at the end of the push-rod applies even pressure to the soil core bottom. The frame can be bolted to one of several settings along the paper cutter, thus, increasing the cutting surface for repeated use.

An extremely sharp, sturdy blade is essential for obtaining thin sections. The blade is torqued against the edge of the paper cutter; thus, lateral movement of the blade is minimal. The bevel of the blade is to the outside, and the flat, inner surface is also torqued against the edge of the metal insert during sectioning. The blade is frequently sharpened with a honing stone and, as a safety precaution, is stored when not in use.

Sections taken from soil cores were placed in numbered petri dishes, filled with water, and examined under a dissecting microscope (maximum magnification = 90×). Optimum lighting was obtained by dual, cross illumination.

3. Results and discussion

3.0. Prefatory note

Sections of litter as thin as 1 mm were obtained, and roots with diameters of 1 cm were cleanly sectioned without destroying sample integrity. Thicker sections of mineral soil (3 mm) were sliced to prevent small rocks from shearing the sample. Larger rocks were removed with forceps to prevent damage to the sections. On an average, a 5 cm core was sectioned into 25 slices.

3.1. Applications of soil sections

The major purpose of embedding and sectioning litter and soil has been to observe the vertical distribution and microhabitats of soil organisms (Haarlov & Weis-Fogh 1953; Anderson & Healey 1970). The simplified technique presented here is adequate for this type of study. Some movement of litter and soil particles occurs around the periphery of the core. This problem can be partially corrected by using a smaller mesh size. In vertical distribution studies, however, the validity of recording individuals found along the periphery of the sample is questionable.

The ultimate goal of the technique is to preserve and view the biota *in situ*. The slow application of a vacuum reduces the possibility of displacement of biota during the embedding process. Ability of the gelatine to infiltrate the sample is a function of core size, amount of vacuum, and size of soil pores. Sometimes the gelatine did not completely infiltrate to the center of the samples, probably due to the limitations of the laboratory vacuum and/or small pore space. Such sections are held intact by gelatine around the periphery of the core and are still viewable under the dissecting microscope. However, microarthropods can float out of such samples when placed in water. Samples taken from sites containing large quantities

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Figure 1. Equipment used in obtaining and embedding samples. a. Coring tool. b. Metal insert. c. Wire mesh insert. d. Soil sample ready for embedding. e. Embedding jar.

Figure 2. Sectioning device. a. Sample holder (aluminium insert). b. mounting block. c. Aluminium frame. d. Mounting block for push rod. e. Push rod.

Figure 3. Fragment of an Oribatid mite (*Ceratoppia bipilis*) containing a large mass of fungal hyphae.

of wet organic material are more difficult to embed with gelatine. The absence of air spaces in such samples or the presence of large quantities of polyphenols (ANDERSON & HEALEY, 1970) hinders the formation of firm gelatine.

A number of descriptive and quantitative models have been developed to examine the role of soil invertebrates as potential regulators of decomposition processes (e. g. GIST & CROSSLEY 1975a; CROSSLEY 1977; WEBB 1977). Soil sectioning is a very useful, preliminary step in examining the magnitude of potential flows between components of the soil biota. Examination of soil sections under a dissecting microscope biases the observer's attention away from microscopic bacteria, protozoans and smaller nematodes, but emphasizes the presence of microarthropods, annelids, feces, exoskeletons and fungi. One current hypothesis developed as a result of examinations of soil sections concerns the contribution of nutrients from invertebrate remains to soil fungi (Figure 3). Both fungi and certain invertebrates, especially Oribatid mites, Diplopoda and Pulmonata, are known for high concentrations of certain nutrients such as calcium (CROMACK et al. 1977; GIST & CROSSLEY 1975b; REICHLE et al. 1969). Invertebrate remains may supply a substantial proportion of nutrients to fungi. Future investigations of fungal-invertebrate interactions should include decomposition studies of arthropod remains of different nutrient quality, as has been done with chitin (OKAFOR 1966). Embedded sections of litter containing these arthropod remains will provide a visual perspective to more quantitative analyses.

The sectioning device may also be used with frozen rather than embedded samples. Attempts are presently being made to sub-sample both embedded and frozen sections for scanning electron microscopy and recording the corresponding elemental distribution on the EDAX (Energy Dispersion X-Ray Spectrometry).

3.2. Limitations of sections

While soil sections can be used to demonstrate vertical distributions of microarthropods, the use of this technique to quantify numbers of these organisms is too time consuming. At least 8 hours are required to scan 25 sections taken from a single core. Smaller microarthropods may be overlooked. Counts of larger microarthropods, particularly Oribatid mites, are complicated by the presence of undecomposed exoskeletons. Thus, the use of embedded sections to quantify microarthropod populations is not justified given the availability of other, less time-consuming and less biased methods (e.g. EDWARDS & FLETCHER 1970).

4. Summary · Zusammenfassung

A simplified procedure for embedding and sectioning litter and soil samples based on the procedure of ANDERSON & HEALEY (1970) but using inexpensive and common materials and equipment is presented. Litter and soil sections made by this method are useful in providing a visual perspective not attainable by Tullgren or flotation extractions. Embedded sections are particularly useful in observing vertical stratification of biota, and evaluating potential interactions between microflora, fauna, and soil composition and structure.

Eine vereinfachte Gelatine-Einbettungstechnik zum Schneiden von Streu- und Bodenproben

Es wird ein vereinfachtes Verfahren zur Einbettung und zum Schneiden von Streu- und Bodenproben vorgestellt, das aus dem von ANDERSON & HEALEY (1970) beschriebenen Verfahren basiert, doch zu dessen Durchführung es nur billiger Materialien und Gerätschaften bedarf. Streu- und Bodenschnitte, die nach dieser vereinfachten Methode hergestellt wurden, vermitteln einen visuellen Einblick, wie er indirekt mit Hilfe der Tullgrenauslese oder von Flotationsmethoden nicht erlangt werden kann. Eingebettete Schnitte sind insbesondere nützlich, um die vertikale Stratifikation der Biota und die potentiellen Interaktionen zwischen Mikroflora, Fauna, Bodenzusammensetzung und -struktur zu bonitieren.

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Address of the authors: T. R. SEASTEDT and D. A. CROSSLEY, Ir., Department of Entomology and Institute of Ecology, University of Georgia, Athens, Georgia 30602, U.S.A. and ARUNA KOSHARI, Department of Botany, Baranas Hindu University, Ind — 221005 Varanas, India.

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